Poster

In vitro genome editing for testing potential gRNAs in CRISPR/Cas9 strategy

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ABSTRACT

Motivation: CRISPR/Cas9 is a promising strategy that improves the efficacy of homology recombination, opening a wide number of possibilites for genome editing. The use of this technology on the reparation of single nucleotide mutations is being under investigation to recover the phenotype of a number of diseases, as the Crigler-Najjar syndrome.

Methods: The genomic target sequence of the Ugt1a1 mouse gene was cloned into vectors that were designed to assess efficiency in the generation of double strand brakes (DSB) by engineered nucleases. We have designed different sgRNAs targeting this genomic region. The vectors were transfected into Hek293 together with a plasmid expressing the Cas9 nucelase and the sgRNA, generating a DSB in the target sequence. After recombination, the inactive luciferase gene recovers activity, which is proportional to the cutting efficiency of the nuclease. Then, luciferase and T7 assays were used to determine the activity of gRNAs to target the specific genome locus.

Results: Depending on the DNA sequence where is located the gRNA and, specially, the PAM sequences, we have obtained different results on the activity of our gRNAs. However, different factors, as the type of cells transfected or the vectors used, can affect the final activity of the gRNAs and thus, the whole CRISPR/Cas9 activity.

Conclusions: With this project, it has been demonstrated the importance of a good design and optimization of the protocols to choose the most efficient gRNAs for CRISPR/Cas9 machinery, as well as in the vectors used to synthetize all the components needed.

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